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Report No. IITRI-L6023-16
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

Contract No. NASr-22

National Aeronautics
and Space Administration
Washington, D.C.

IIT RESEARCH INSTITUTE

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December 1, 1968 through February 28, 1969

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I. INTRODUCTION

Current experiments are investigating the effects of daily freeze-thaw cycles and ultraviolet irradiation on the survival of bacteria airborne in dust clouds of soil. Representative types of bacteria present in spacecraft assembly areas or normally indigenous to humans were used: Bacillus cereus, B. subtilis, Escherichia coli, and Staphylococcus aureus.

These organisms were inoculated into a limonite coated soil (collected in the Sonoran Desert, Arizona) and were irradiated daily with a Mars daily total equivalent ultraviolet flux over the 2400 to 2800 A region: a value of 298 μWcm^{-2}. Concurrently, the organisms were also exposed to a daily freeze-thaw cycle of 16-hr at -65°C and 8-hr at 30°C.

The decrease in viable cell counts of B. cereus, B. subtilis, and S. aureus was similar to the results from experiments without ultraviolet irradiation and daily freeze-thaw cycle. The viable cell count of B. cereus and B. subtilis decreased 99%

and S. aureus decreased 99.9% while suspended in simulated Martian dust clouds for 21 days. E. coli was extremely sensitive to the test environment. Viable cells were not recovered after 1 day in the test environment.

The data indicates that the ultraviolet flux at the surface of Mars may not be as effective a sterilizing agent as previously assumed.

The decreased viability does not, at this time, indicate any relaxation of spacecraft sterilization constraints since significant numbers of bacteria would survive from initial populations and still threaten the contamination of Mars by earth organisms.

The following presentation and abstract dealing with work performed under Contract NASr-22 were made during the report period.

a. Semiannual project report presented at the Sterilization Technology Seminar, February 11 and 12, held at Cape Kennedy, Florida.

b. An abstract of the paper entitled "Effect of Ultraviolet on the Survival of Bacteria Airborne in Simulated Martian Dust Clouds," was submitted to the National Academy of Sciences for presentation at the 1969 Annual COSPAR Meeting. We have since been informed of its acceptance by the NAS Space Science Board. The paper has been referred to the International Working Group V Space Biology.

II. EXPERIMENTAL METHODS

A. Chamber

A rotating chamber, similar to the one previously described in Report No. IITRI-L6023-13 and -14, has been constructed of aluminum 12-in. in diameter and 12-in. long. The new chamber was required because of the unacceptable performance of the first chamber at -65°C.

A baffle system of screen material creates dust clouds of the soil as the chamber rotates at approximately 16 rev/min. The rotating chamber is presently operated in an environmental chamber which is programmed for daily freeze-thaw cycle of 16-hr at -65°C and 8-hr at 30°C. Irradiation with an ultraviolet lamp is for 1-hr at the beginning of the thaw portion of the daily freeze-thaw cycle.

At one end of the chamber there is a suprasil quartz window set in the face plate. Suprasil transmits 90% of the ultraviolet over the 2000 to 3000 A region with the transmittance dropping off sharply at 1600 A. A sampling port is present in the face plate at the opposite end together with a port that permits evacuation of the chamber to partial pressures equal to those currently estimated for the Martian barometric pressure. At present, the chamber is being modified so that soil samples can be removed without disrupting the chamber environment when operating at partial pressures less than 760 mm.

The chamber's capacity is several pounds of soil. The type of soil presently used is a limonite coated soil from the Sonoran Desert in Arizona.

The entire chamber together with the soil is autoclaved at 121°C for 1 hr on each of two succeeding days and dried for 1/2-hr in a vacuum autoclave. After cooling, the soil is inoculated by spraying 4-ml of a phosphate buffer suspension of the organisms calculated to give 10^6 viable cells/g of soil without introducing excessive amounts of water.

B. Ultraviolet Source

The ultraviolet lamp is a Hanovia type A lamp (No. 673A, 550 watts) with a 4.5-in. arc length that, at a distance of 27-cm, emits approximately 8 times the ultraviolet intensity (over the 2400 to 2800 A region) of that calculated for the surface of Mars.

The lamp intensity, or irradiance, for different wavelengths in the ultraviolet region was calculated with an Eppley thermophile (Eppley Laboratory Equipment Co., Newport, Rhode Island). The thermophile is a circular, 12-junction, bismuth-silver instrument with a sensitivity ($\mu V/\mu Wcm^{-2}$) of 0.15 and a response time of 1.5 sec. Thermophile, or radiometer, readings were taken at different distances from the lamp with various filters to obtain the lamp's spectral characteristics. Approximately 50% of the lamp's emission is

in the ultraviolet region (2200 to 4000 A) with about 10% in the 2400 to 2800 A region.

To determine the daily operating time of the lamp the cumulative value of the solar irradiance above the Earth's atmosphere over the 2400 to 2800 A region was multiplied by 0.46 to give the total daily equivalent irradiance at the surface of Mars which was calculated to be 298 μWcm^{-2}. Radiometric measurements showed an irradiance of 2600 μWcm^{-2} at the chamber front (27-cm from the ultraviolet source) and 530 μWcm^{-2} at the back of the chamber (59-cm from the ultraviolet source). To obtain the Martian equivalent for these irradiances the lamp would have to operate 0.92-hr (55 min) and 4.4-hr (264 min) respectively. It was also found by radiometric measurements that the dust cloud decreased the ultraviolet irradiance at least 13-fold since the reading was below the threshold of the potentiometer.

The initial experiments used a 1-hr daily ultraviolet exposure. Subsequent experiments will study increased exposure times to determine any apparent effect.

C. Preparation of Bacterial Cultures

Spores of *B. cereus* and *B. subtilis* were produced on trypticase soy agar (BBL). After incubation at 35°C for 6 to 7 days, free spores comprised 99% of both cultures. The spores were harvested in chilled 0.025 M phosphate buffer (pH 7.0) and

washed seven times before final suspension in the buffer. The suspensions were centrifuged at 5°C in an International refrigerated centrifuge, model PR-2, with a No. 284 head, at 2,600 rev/min (1,500 x g). The suspensions were stored at 5°C and used without heat shock since the germination of spores by soil particle abrasion was being investigated.

Stock cultures of E. coli and S. aureus were produced by growing the organisms on the surface of trypticase soy agar for 24 or 48-hr at 35°C. Harvesting and storage procedures were the same as previously described for B. cereus and B. subtilis.

D. Bacterial Recovery and Enumeration

Soil samples were aseptically removed at various time intervals (usually at 1- and 3-hr post inoculation and at 1, 3, 7, 14, and 21 days) through the sampling port while the chamber rotated.

The samples were diluted with 0.1% peptone water and appropriate dilutions were plated on trypticase soy agar to recover viable bacteria (total counts). Spore counts were performed by heating the soil suspension dilutions for 10 min at 80°C before plating on trypticase soy agar. The plates were incubated at 37°C for 24 or 48-hr. Bacterial counts are reported as the average count of duplicate plates from each of two or three samples.

Other recovery media were used concurrently as either differential (purple agar base with mannitol incubated anaerobically and MacConkey's agar) or minimal (basal salts agar) media to either enhance recovery of a particular species or to detect metabolically injured cells.

III. RESULTS AND DISCUSSION

No attempt was made to control the relative humidity in the rotating chamber which would fall to 0% during the freeze period and would assume ambient room relative humidity during the thaw period. The daily freeze-thaw condition resulted in the slight loss of moisture. A soil moisture determination showed the moisture content of the soil to be 2.4% and 1.6% after 3-hr and 7 days, respectively.

Mechanical difficulties with the refrigeration system of the environmental chamber did not allow the temperature to be maintained at -65°C during the frozen portion of the daily freeze-thaw cycle. The temperature fluctuated between -18 and -40°C for the first several days. This is a very critical temperature range (0 to -45°C) for bacterial cells and could have a greater adverse effect on cell viability than would occur at a constant -65°C.

E. coli did not survive a single freeze-thaw/ultraviolet exposure. The ultraviolet or freeze-thaw effects on cell viability cannot be determined since the same results were obtained with a similar organism, Serratia marcescens, at room temperature without ultraviolet irradiation.

The viable cell count of S. aureus decreased approximately 99% during the initial 3-day exposure to the environmental conditions while B. cereus and B. subtilis decreased 75 and 80%, respectively. These data are shown in Figure 1. By the end of the 21-day exposure period the viable cell count of B. cereus and B. subtilis decreased to 99% while S. aureus decreased to 99.9%.

There was a difference in the decrease rate of cell viability between the cells exposed to a daily freeze-thaw cycle and ultraviolet irradiation and those cells exposed to room temperature without ultraviolet irradiation. The cell viability of B. cereus and B. subtilis exposed to room temperature (IITRI Report No. L6023-15) decreased 99% over the initial 7-day period without any further reduction whereas the cell viability of these same organisms exposed to daily freeze-thaw and ultraviolet irradiation decreased 75 and 80%, respectively, during a similar period with a subsequent decrease during the remaining 14 days which brought the cell populations to similar levels in both sets of experimental conditions.

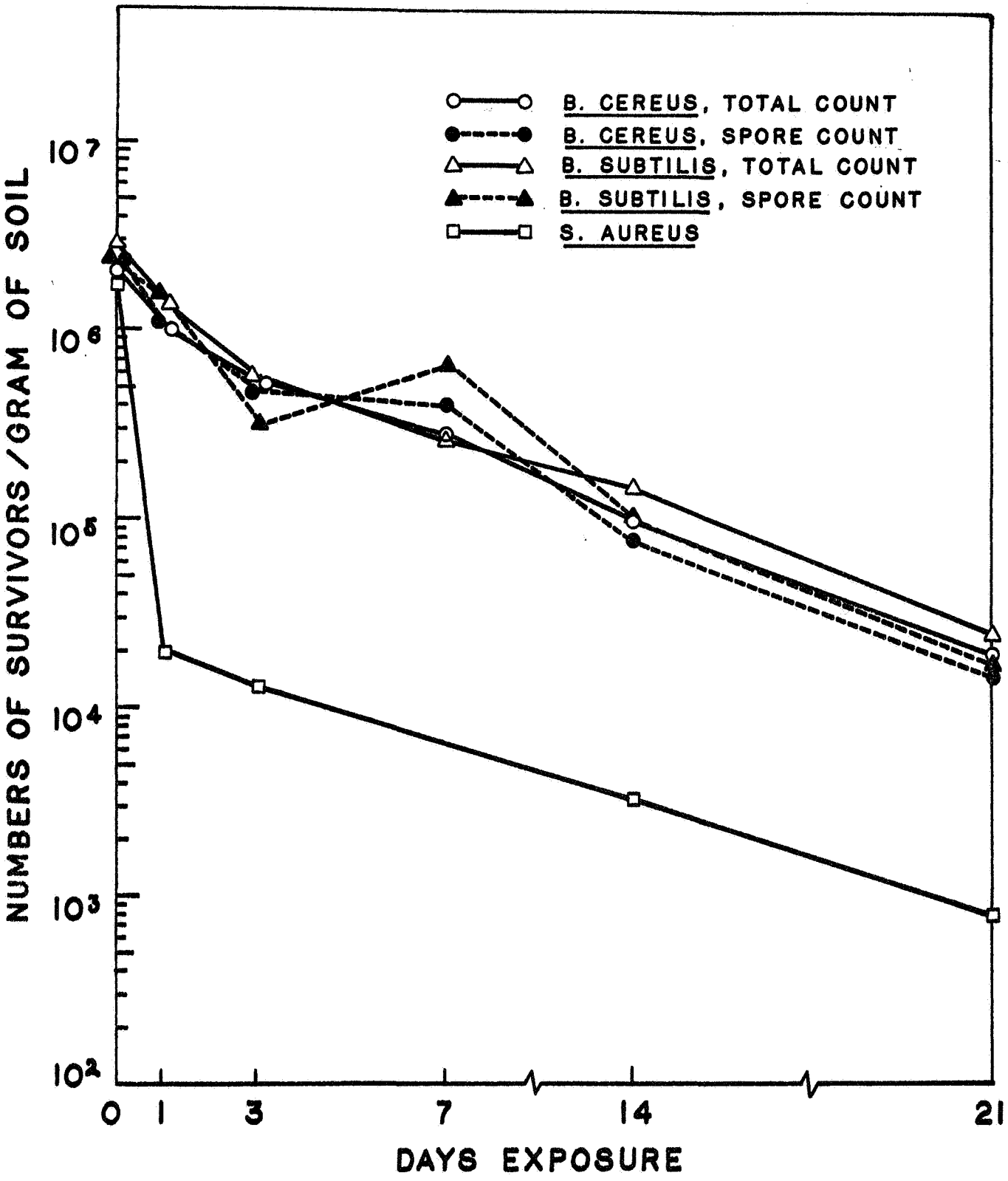

FIG. 1 THE EFFECT OF ULTRAVIOLET IRRADIATION AND DAILY FREEZE-THAW ON THE SURVIVAL OF SELECTED BACTERIA IN SIMULATED MARTIAN DUST CLOUDS.

The later decrease in cell viability of the freeze-thaw and ultraviolet exposed organisms could have resulted from thermal sensitization or it could have been the result of ozone toxicity. Both factors are presently being investigated. The thermal sensitization is of two types:

1. The temperature fluctuating between -18 and -40°C instead of the programmed -65°C
2. The temperature rise from operating the ultraviolet lamp.

The 7-day S. aureus survivors are not shown in Figure 1. This was due to a difficulty in recognizing S. aureus colonies in the presence of large numbers of B. cereus and B. subtilis. The difficulty was corrected by reincubating subsequent plates for 48-hr after pigmentation of S. aureus was more pronounced.

IV. SUMMARY

The viable cell count of B. cereus and B. subtilis decreased 99% and S. aureus decreased 99.9% during a 21-day exposure to simulated Martian dust clouds with daily freeze-thaw cycles and ultraviolet irradiation.

The data indicates that ultraviolet irradiation at the surface of Mars is not the lethal agent suggested by some biologists and that the surviving organisms still present a problem to the prevention of the contamination of Mars.

Confirmation of the reported experiments as well as additional experiments with other environmental conditions such as moisture concentration, gaseous composition, and barometric pressure will be continued.

V. PERSONNEL AND RECORDS

The experiments were planned with the counsel of Dr. E. J. Hawrylewicz and the technical assistance of Mr. B. T. Anderson and Mrs. M. L. Cephus.

Experimental data are recorded in IITRI Logbooks C19044 and C19157.

Respectfully submitted,

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